Innovative Food Science and Emerging Technologies 11 (2010) 123–129

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Thermal inactivation of *Bacillus anthracis* Sterne in irradiated ground beef heated in a water bath or cooked on commercial grills ☆

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ARTICLE INFO

Article history:
Received 18 March 2009
Accepted 17 August 2009

Editor Proof Receive Date 5 September 2009

Keywords:
Bacillus anthracis
Ground beef
Pathogen
Biosecurity
Food safety

ABSTRACT

The thermal stability of heat-shocked and non-heat-shocked spores of the virulence-attenuated Sterne strain of *Bacillus anthracis* was evaluated at select temperatures in irradiated, raw ground beef (25% fat) heated in a water bath or cooked using two different commercial grills. For the former, 3-g portions of inoculated ground beef were packaged in bags that were completely immersed in a temperature-controlled circulating water bath held at 65 °C (149 °F), 70 °C (158 °F), 75°(167 °F), and 80 °C (176 °F) for a predetermined length of time. For the latter, formed ground beef patties (95-g each) were inoculated with spore stock A or B of the Sterne strain and then cooked on a commercial open-flame gas grill or on a commercial clamshell electric grill to achieve target internal temperatures of either 71.1 °C (160 °F), 82.2 °C (180 °F), or 93.3 °C (200 °F). Cooking ground beef patties on commercial grills, resulted in reductions of ca. 0.8 to 3.5 $\log_{10}$ CFU/g for spore stocks A and B of *B. anthracis* Sterne after heating to 71.1 °C (160 °F), 82.2 °C (180 °F), or 93.3 °C (200 °F) on either the open-flame gas grill which required ca. 9.6 min to reach the target internal temperatures or on the clamshell electric grill which required ca. 4.0 min to reach the target internal temperatures. In comparison, our data using a water bath system and heating at 65° to 80 °C predict nearly 4 log reductions in spore levels for short times, ~½ min, depending possibly on the temperature. Thus, our data suggest that models based on heating ground beef in a water bath is not a good predictor of reductions of levels of spores of *B. anthracis* Sterne strain that would be obtained when cooking ground beef patties on commercial grills under conditions that may be typically used by consumers and/or retail establishments. Nevertheless, our data validated that cooking ground beef patties on a commercial grill at a temperature considered to be "well-done" and a temperature (71.1 °C;160 °F) recommended by the USDA/FSIS, is effective at killing spores of *B. anthracis* Sterne.

Industrial relevance: Heating ground beef in a water bath or cooking ground beef patties on commercial grills under conditions simulating those that are used by consumers and/or that occur in retail food service establishments is effective at killing spores of *B. anthracis* Sterne.

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1. Introduction

Bacillus anthracis causes a disease known as anthrax, which is an often fatal bacterial infection that occurs when spores enter the body through abrasions in the skin or by inhalation or ingestion. Thus, the disease could be of the cutaneous, inhalation, or gastrointestinal forms (Friedlander, 1999). Symptoms of gastrointestinal infection or food-borne anthrax include nausea, fever, abdominal pain, diarrhea, ulceration, hemorrhage, edema, and ascites, as well as an eventual fluid shift from the vascular compartment leading to shock and death of an individual within 2 to 3 days following consumption of a contaminated food (Sirisanthana & Brown, 2002). Therefore, contamination of food with *B. anthracis*, although rare, is a potentially significant public health hazard.

Since the American food chain relies on a centralized production and processing system for an ever-increasing and widespread distribution of food products (Sobel, Khan, & Swerdlow, 2002), our food supply is particularly vulnerable to deliberate contamination with threat agents. Although the incidence of *B. anthracis* in foods is rare and although medical advances have decreased its importance for both livestock and humans (Smith et al., 2001), following the October 2001 incident of terrorism involving the purposeful addition of *B. anthracis* spores to mail, this pathogen has been identified as a potential biological weapon that could be used by terrorists. An intentional contamination of food

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1466-8564/$ – see front matter. Published by Elsevier Ltd
doi:10.1016/j.ifset.2009.08.011

with *B. anthracis* spores could result in public panic, massive fatalities, and large economic losses. Thus, in a previous study we evaluated the fate of *B. anthracis* spores to germinate and/or survive in ground beef, a high-volume, minimally-processed food (Tamplin, Stewart, Phillips, Luchansky, & Kelley, 2008). Our results showed that *B. anthracis* was weakly inactivated at temperatures between 2 and 16 °C and at temperatures ≥45 °C, whereas growth was observed when ground beef was stored at temperatures ranging from 17 to 44 °C (Tamplin et al., 2008). Therefore, every effort should be made to guard against the introduction of this pathogen into foods, be it from natural contamination or from purposeful addition, and to validate the efficacy of existing processing technologies to eliminate it.

At present, there is a general lack of information concerning the effectiveness of interventions that would eliminate *B. anthracis* spores in food. The use of heat to achieve a specific lethality is the most important critical control point used to assure the microbiological safety of processed foods, and it plays a major role in preventing foodborne disease. Numerous studies have been published, before and after 2001, on the resistance of spores of *B. anthracis* and/or its potential surrogates to heating in a variety of menstrums, including distilled water, physiological saline, synthetic media, skim milk, phosphate buffer (pH 7.0 or pH 4.5), and orange juice (Francis, 1956; Montville, Dengrove, De Siano, Bonnet, & Schaffner, 2005; Murray, 1931; Novak, Call, Tomasula, & Luchansky, 2005; Schneiter & Kolb, 1945; Stein & Rodgers, 1945). From these studies, it is clear that *B. anthracis* spores are not unusually heat resistant compared with other *Bacillus* species and/or other *B. anthracis* strains; D values, that being the time to achieve a 90% or 1-$\log_{10}$ reduction, for *B. anthracis* strains Sterne, Vollum, and Pasteur in buffer, milk, or orange juice ranged from <1 min at 90 °C to >200 min at 70 °C (Montville et al., 2005). Of note, spores of the avirulent Sterne strain of *B. anthracis* exhibited similar heat resistance to that of other *Bacillus* species, including virulent strains of *B. anthracis* (Montville et al., 2005).

Our search of the literature found no studies on the heat resistance of *B. anthracis* in solid foods. There are, however, numerous studies published on the fate of other spore-forming food borne pathogens in response to thermal challenge in a solid food. For example, Byrne, Dunne, and Bolton (2006) reported D values of 32.1 and 2.0 min at 85 °C and 95 °C, respectively, for a three-strain cocktail of *Bacillus cereus* spores (strains DS 4313, DSM 626, and NCTC 07464) inoculated into pork luncheon roll. Faille, Lebret, Gavine, and Maingonnat (1997) also evaluated the heat resistance of *B. cereus* spores (strains LMG 6923, CUETM 93/60, CUETM 93/61, and CUETM 93/62) in mechanically separated poultry meat and reported D-values that ranged from 3.3 to 209 min at 80 °C, 5 to 89 min at 85 °C, and 2 to 17 min at 90 °C. As another example, Juneja, Eblen, Marmer, Williams, Palumbo, & Miller (1995) explored the possibility of predicting the heat resistance of non-proteolytic *Clostridium botulinum* spores in turkey slurry (50% in H_2O) from data obtained in phosphate buffer (0.1 M, pH 7.0), and found increased thermal resistance in the slurry compared to buffer. As reported by others, the thermal resistance of spores of *Bacillus* species can also be appreciably increased by food components such as protein and fat (Behringer & Kessler, 1992; Shehata, Khalaffalla, El-Magdoub, & Hofi, 1977). Thus, it would be misleading to predict the thermal-death-time (TDT) values of *B. anthracis* spores in ground beef from data obtained in buffer, milk, or any other liquid substrate. Accordingly, the work reported herein was undertaken to provide a quantitative and comparative assessment of the heat resistance of spores of *B. anthracis* Sterne in ground beef heated in a water bath and cooked on commercial grills. Based on the D-values for *B. anthracis* strains in buffer (pH 7.0 or pH 4.5), pasteurized 2% fat milk, or pasteurized orange juice reported by Montville et al. (2005), the attenuated Sterne strain was selected as a suitable surrogate for virulent *B. anthracis* strains, including the Ames strain. The results presented herein could be used to establish cooking temperatures and times that would minimize the potential danger of *B. anthracis* infections from the gastrointestinal form of anthrax arising from its intentional addition to foods such as ground beef that are produced in high volume and subsequently cooked and consumed by a significant portion of the population.

2. Materials and methods

2.1. Bacterial strains and ground beef

Two different spore stocks of the avirulent Sterne strain of *B. anthracis* were used in this study to address the potential for variability among sources of spores/strains. One of the spore stocks, designated as spore stock A, was obtained from the United States Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) Microbial Outbreaks and Special Projects Laboratory (MOSPL) in Athens, Georgia. The MOSPL spore stock was originally obtained from Dr. Bruce Harper of the U.S. Army Dugway Proving Grounds (Dugway, UT). The other spore stock, designated as spore stock B, was originally obtained from Drs. Jeff Karns and Michael Perdue ((Perdue, Karns, Higgins, & van Kessel, 2003); USDA, Agricultural Research Service, Beltsville Area Research Center, Beltsville, MD). The spores were prepared essentially as described by Novak et al. (2005) and were stored in 20% ethanol (vol/vol) in dH_2O at 4 °C. Spore population densities were determined by plating serial dilutions in 0.1% (wt/vol) peptone water, with and without prior heat shock (75 °C for 20 min), onto brain heart infusion (BHI) agar plates. It is worth mentioning that heat shocking activates the spores to germinate. Therefore, higher numbers are likely to be enumerated on recovery medium. Data were recorded as colony forming units (CFU)/ml after incubating the plates at 37 °C for 24 h. Fresh raw ground beef (75% ± 1% lean) was purchased at a local grocery store and was prepared, irradiated, and stored essentially as described by Porto-Fett, Juneja, Tamplin, and Luchansky (2009).

2.2. Thermal inactivation of *B. anthracis* Sterne in irradiated ground beef heated in a temperature-controlled water bath

Fifty gram portions of irradiated ground beef were inoculated with an appropriate dilution of non-heat-shocked *B. anthracis* Sterne stock A spores to achieve a target level of ca. 7.0 $\log_{10}$ spores/g. The inoculated ground beef samples were mixed and aseptically weighed into 3-g portions as described (Juneja, Marks, & Mohr, 2003; Porto-Fett et al., 2009). Unheated ground beef inoculated with non-heat-shocked spores served as a control. Thermal inactivation was conducted by placing the inoculated samples in a basket that was completely submerged in a temperature-controlled, circulating water bath (Exacal, Model Ex-251HT, NESLAB Instruments Inc., Newington, NH) stabilized at 65 °C (149 °F), 70 °C (158 °F), 75°(167 °F), or 80 °C (176 °F) ± 0.5 °C (0.4 °F) as previously described (Lindsay & Murrell, 1985; Porto-Fett et al., 2009). The temperature of the water was monitored using a type K thermocouple (Omega Engineering Inc., Stamford, CT) connected to a temperature data logger (Dickson SP150, Addison, IL). Total heating times ranged from 180 min at 65 °C (149 °F) to 5 min at 80 °C (176 °F). After removal from the water bath, samples were immediately plunged into an ice–water bath and analyzed within 30 min. For each of three trials, duplicate samples were analyzed at each sampling interval. The data are reported as the average $\log_{10}$ CFU/g for all trials and replicates.

2.3. Thermal inactivation of *B. anthracis* Sterne in irradiated ground beef cooked on commercial grills

Irradiated ground beef was transferred aseptically to a sterile stainless steel food-processing bowl and inoculated with spores that were heat-shocked in sterile 0.1% peptone water at 80 °C for 10 min or with non-heat-shocked spores of spore stock A or B of *B. anthracis* Sterne to achieve a target level of ca. 6.0 $\log_{10}$ spores/g of meat. Thereafter, the inoculated ground beef was mechanically mixed

(KitchenAid, Model KP26M1XBS, St. Joseph, MI) for 2 min at room temperature (22 °C ± 1 °C or 71.6 °F ± 1.8 °F) to more evenly distribute the inoculum throughout the meat. Ground beef patties were formed, stored, and subsequently cooked on both a commercial open-flame gas grill (Model XXE-4, Baker's Pride, New Rochelle, NY) or on a commercial clamshell electric grill (Model GR14, Star Manufacturing International Inc., St. Louis, MO) to achieve target internal temperatures of either 71.1 °C (160 °F), 82.2 °C (180 °F), or 93.3 °C (200 °F) essentially as described by Porto-Fett et al. (2009). In addition, the purge from each patty at each cooking temperature was collected by placing small aluminum foil containers directly below each patty on the grill bottom or in the grease drawer for hamburgers cooked on an open-flame gas grill or on a clamshell type grill, respectively. For each of three trials, three ground beef patties were cooked at each of the three temperatures tested for each grill type.

2.4. Enumeration of surviving B. anthracis from ground beef

Surviving spores of *B. anthracis* Sterne were recovered from heated ground beef similar to the protocol we used in a companion study to recover *Yersinia pestis* from heated ground beef (Porto-Fett et al., 2009), with the exception that surviving spores were enumerated on brain heart infusion (BHI) agar plates (Difco Laboratories Inc., Detroit, MI). To authenticate the identity of *B. anthracis*, typical colonies were plated onto Anthracis Chromogenic selective/differential agar (R&F Laboratories, Downers Grove, IL) and the biochemical reactivity was verified by using API 50CH test strips (BioMérieux Laboratories, St. Louis, MO). The plates were incubated at 37 °C for 16 to 20 h before counting the survivors.

2.5. Data analyses

Inactivation studies in ground beef heated in a water bath were conducted to provide a simple mathematical description of the *B. anthracis* Sterne survival curves. To accomplish this, observed survival curves on the logarithmic scale, $R(t) = \log_{10}(S(t))$ versus time, t, where $S(t) = N(t)/N(0)$ is the proportion of surviving cells, or the relative reduction at time t, and $N(t)$ and $N(0)$ are the levels of spores per gram at time t and zero, respectively, were plotted and characterized. Appropriate functions with the identified characterizations were then used to fit the observed data. Analyses were performed using the SAS software system (SAS, 2004); measurements made at the same time were considered as "blocks" thus mixed effect models with random blocks effects, using maximum likelihood estimation, was used to estimate parameter values. To compare models for goodness of fit, the AIC criterion was used. For a secondary model, seemingly unrelated regression (SUR) was used to estimate values for parameters. The phrase "lethality at time t" is used to denote the negative of the $\log_{10}$ of the relative reduction at t: that is, $\text{Leth}(t) = R(t)$.

The heat resistance data obtained from cooking patties using two different commercial grills were analyzed by analysis of variance (ANOVA) using SAS (2004) to determine if there were statistically significant differences among the treatments and spore preparations. The Bonferroni mean separation test was used to determine significant differences ($P < 0.05$) among means and/or among treatments or strains.

3. Results and discussion

3.1. Inactivation of B. anthracis in ground beef heated in a temperature-controlled water bath

In preliminary experiments comparing the heat resistance of *B. anthracis* Sterne there were no significant differences ($P > 0.05$) between heat-shocked (75 °C for 15 min) and non-heat-shocked preparations of spore stock A (data not shown). Therefore, only non-heat-shocked spores were used for subsequent experiments when heating inoculated ground beef in a water bath.

All the observed survival curves ($\log_{10}$ relative reduction versus time) for spore stock A of *B. anthracis* Sterne obtained were asymptotically convex (Fig. 1). Fig. 1 shows plots of data from time = 0 to time = 0.5 min, leaving out data points at larger times for purposes of seeing clearly the relatively rapid decline before the nearly literal "leveling" of the levels for larger times. In other words, the "tails" of the survival curves were nearly parallel to the x-axis, above zero, for long periods (for times up to 3 h beyond the times shown in Fig. 1). We interpret this to mean that for a given temperature, there appears to be a subpopulation of heat resistant spores not being inactivated rapidly over time. These properties eliminate some commonly used survival models such as those based on the Weibull distribution. For these $R(t) = -at^b$, where values of a and b are estimated from data, no such non-zero asymptotic behavior occurs. Furthermore the ratio of the $R(t)$ to the derivative of $R(t)$ is equal to t/b, thus a plot or regression of this ratio divided by t should be independent of t, and equal to a constant $1/b$. However, further data analyses (not presented) did not indicate such a relationship; generally the ratio was positively correlated with time.

A function which could satisfy better the above properties is the Gompertz function,

$$g(t) = A - C\exp(-\exp(-bt + m)) \quad (1)$$

for parameter values A, C, b and m, where $b > 0$ to ensure a negative derivative and asymptotic convexity; for $b > 0$ the function is convex for all $t > 0$ if and only if $m < 0$. The asymptotic lethality (as t gets large) is $C(1 - \exp(-\exp(m)) > 0$.

The problem with the Gompertz function is that the parameters do not have a readily, or conventional, microbiological interpretation. This hampers generalizing the estimates over different temperatures as well as providing simple descriptions that others are familiar with. Our attempt was to use a model which would roughly serve as a convenient and ideally conventional way of describing lethality even at the expense of some accuracy. For example, using D-values to help describe survival curves, even when the properties of them are strictly inconsistent with the properties of the survival curve, can be useful because the inaccuracies with such a description are not great or misleading. In our situation, clearly using single D-values to describe the observed survival curves would be misleading; however,

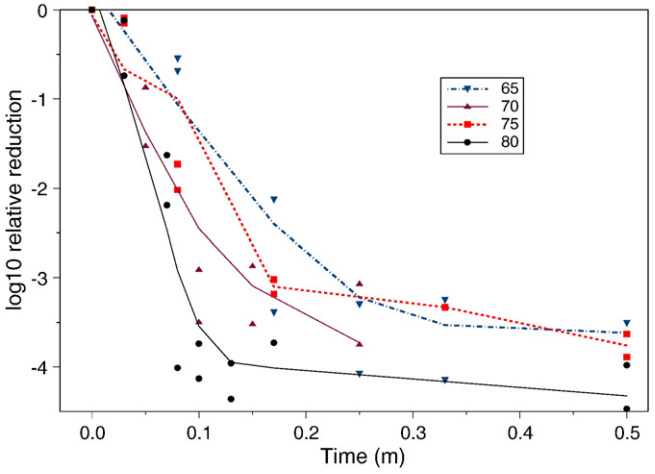

Fig. 1. Plot of observed survival curves, of *Bacillus anthracis* in ground beef heated in a thermostatically controlled water bath, and fitted spline curves using S-Plus smoothing options, for each temperature. Shown are observations up to 0.5 min. The dashed line and faced down (apex down) triangle are for 65 °C (149 °F); the light solid line and the faced up triangle are for 70 °C (158 °F); the dotted line and squares are for 75°(167 °F); and the dark line and circles are for 80 °C (176 °F).

describing the survival curves as comprising two regimes (regions of time), each with their own *D*-value, might not be misleading, at least before data analysis would demonstrate otherwise.

Thus, in addition to fitting a Gompertz equation, a bi-phasic survival curve, estimated using spline regression, was used to describe the observed $\log_{10}$ reductions. For each replicate the outputs from this model are two *D*-values, one for each regime from which *z*-values could be computed, and a point of intersection (knot) of the two regimes. The requirement imposed is that the fitted survival curve must be continuous.

To confirm our data analytical observations given above, each model was fit using a mixed-effect, non-linear model, as described in the data analysis Section 2.5. The Weibull function has two fixed parameters; the Gompertz and spline functions each has three parameters. Table 1 presents the comparative goodness-of-fit statistics using AIC (Akaike's information criterion) values for the obtained fits. The AIC statistic adjusts the likelihood ratio statistic by taking into account the number of parameters being fit and is a standard output of many statistical packages used for regression analyses; the lower the value the better the fit. In general, the Gompertz function fits better as indicated by the smaller AIC values, while the Weibull function fits are considerably worse. Table 2 presents some summary statistics of the fits for the spline functions and the Gompertz functions including, for the spline function, D-values, the point of intersection of the two regimes for each temperature, and the estimated $\log_{10}$ reduction at the intersection point, and, for the Gompertz function, the estimated lethality at ½ min and the estimated asymptotic lethalities.

The estimates of log reductions obtained from the bi-phasic model tend to be slightly larger than the observed log reductions when the latter are less than about 1 $\log_{10}$ and slightly larger when the observed log reductions are greater than 4. However, essentially most of the lethality will be achieved within the first 30 s; after that little additional lethality is obtained.

Generalizing the Gompertz parameters values over the 4 temperatures would be difficult because, even after logarithmic transformations, the relationships of the parameter values and temperature do not appear to be linear, primarily because of the estimates of the values at 80 °C. However, thermal death curves (plots of $\log_{10}$ *D*-value versus temperature, Fig. 2) appear not to be inconsistent with the expected linear or near linear relationship of the $\log_{10}$ (*D*-values) versus temperature, even though the linear fit does not appear to be particularly as strong as is normally expected or desired. For the spline model, there are 3 parameters that must be generalized, namely, the two $\log_{10}$ (*D*-values) and the point of intersection of the two regimes. Thus there are 3 equations, which are not unrelated; to fit the parameters, the SUR (seemingly unrelated regression) option was used in the PROC MODEL routine of SAS (2004). For each temperature, the dependent variables were the averages of the $\log_{10}$ *D*-values and the intersection times over the two replicates. It was assumed that the $\log_{10}$ of *D*-values for the first regime was equal to $a_1 + b_1T$, and that for the second was: $a_2 + b_2T$; and the knot time (the time for which the two regimes intersect) was a constant for all temperatures. The estimated *z*-values for the two regimes are thus estimated as: $z_1 = -1/b_1$; and $z_2 = -1/b_2$. The standard errors of these estimated are obtained by approximation, using linear terms of the Taylor series expansions of the ratio: std_error $(z_j) \approx z_j^2$ std_error (b_j). The estimated values of *z* were: $z_1 = 109.7$ °C, with standard error equal to 124.9 °C, while that for regime 2 is: $z_2 = 12.9$ °C, with standard error of 5.9 °C. The relatively high estimated standard errors indicate that accurate predictions of lethality cannot be made using these estimated *z*-values.

Table 1
Goodness-of-fit statistics (AIC statistics) for three models used to fit data for each replicate (Rep).

Temp. °C	Rep	AIC for Weibull	AIC for Spline bi-phasic	AIC for Gompertz
65	1	33.52	16.44	9.44
65	2	31.07	10.78	−0.90
70	1	14.43	10.91	14.91
70	2	37.34	18.08	18.51
75	1	47.16	27.01	29.70
75	2	43.16	21.13	21.08
80	1	37.17	24.68	−0.58
80	2	13.03	1.74	−1.16

A smaller AIC value indicates a better fit.

For practical purposes, since thermal treatments are not applied for longer times than those given in the Table 2, with the probable exception at 80 °C, the results of this study suggest that not more than a 5.0-$\log_{10}$ lethality would be obtained regardless of the temperature of the thermal treatment, at least for temperatures up to 80 °C (176 °F). For the lowest three temperatures, it is reasonable to conjecture that for cooking times likely to be used (>15 s, but less than 2–3 h) the obtained lethalities would likely be between 4–5 $\log_{10}$; though, at 80 °C, more research is needed to determine the lethalities that would be obtained for cooking times greater than 5 min.

Survival curves similar to those observed in this study (Fig. 1) were obtained by Novak et al. (2005) using pasteurized skim milk, inoculated with *B. anthracis* Sterne, that was heated at 100 °C (212 °F) in a water bath system. A rapid decline in the levels of *B. anthracis* Sterne was reported, and thereafter the rate of decline decreased, and the survival curve finally approached a plateau. However, in the region of common temperatures of the Novak et al. (2005) study and our study, the observed and predicted *B. anthracis* spores lethalities reported were quite different: Novak et al. (2005) reported the inactivation of *B. anthracis* Sterne in skim milk as 0.45-$\log_{10}$ CFU/ml after 90 min at 72 °C (161.6 °F), 0.39-$\log_{10}$ CFU/ml after 90 min at 78 °C (172.4 °F), 8.1-$\log_{10}$ CFU/ml after 60 min at 100 °C (212 °F), and 7.7-$\log_{10}$ CFU/ml after 2 min at 130 °C (266 °F). Clearly, the less than 1 $\log_{10}$ inactivation for 90 min at the lower temperatures (72 °C , 78 °C) of the Novak et al. study (2005) were substantially lower than the observed inactivation of 4 $\log_{10}$ for comparable times in our study, covering this range of temperature.

3.2. Thermal inactivation of B. anthracis in ground beef patties cooked using two different commercial grills

The cooking times necessary to achieve internal temperatures of 71.1 °C (160 °F), 82.2 °C (180 °F), and 93.3 °C (200 °F) for ground beef patties cooked on a gas grill were approximately 7.9, 9.5, and 11.5 min (avg. = 9.6 min), respectively, whereas for the electric grill the times were approximately 3.1, 3.9, and 5.0 min (avg. = 4 min), respectively. Thus, the average length of time necessary to achieve these target internal temperatures was significantly ($P<0.05$) longer (about 5.6 min) for patties cooked on a gas grill than for patties cooked on the electric grill. It should also be noted that meat patties can continue to cook even after removal from direct heat. From the time when the patties were removed from the grills until they were chilled/sampled, that being 1.3 min ± 0.5 min, there was an additional increase that ranged from about 6.5 to 10.6 °C in the internal temperature of the patties cooked at 71.1 °C and 82.2 °C, whereas only a slight decrease of approximately 2.8 °C was observed for patties cooked at 93.3 °C on either grill. Thus, regardless of the spore stock or whether or not spores were heat-shocked, for patties cooked on the gas grill, the final average temperatures were 77.6 °C, 92.8 °C and 90.5 °C for target internal temperatures of 71.1 °C, 82.2 °C, or 93.3 °C, respectively. For patties cooked on the electric grill, the final average temperatures were 77.6 °C, 87.8 °C, and 91.8 °C for target internal temperatures of 71.1°, 82.2°, and 93.3 °C, respectively. Thus the difference between the final temperatures for the treatments with the largest two target temperatures is less than the difference of these target temperatures.

Table 2
Estimated *D*-values for each replicate at given temperature and intersection (time) of the two inactivation kinetic regimes.

Temp. °C	*D*-value (min) first regime	*D*-value (min) second regime	Intersection time (min) of regimes	Estimated lethality at intersection time	Estimated lethality ½ m Gompertz	Observed max lethality	Estimated asymptotic lethality Gompertz	max time (min)
65	0.088	442.3	0.35	3.9	3.4	4.4	4.0	180
65	0.050	364.5	0.23	4.6	4.4	4.7	4.5	180
70	0.034	99.0	0.12	3.4	3.5	4.4	3.6	120
70	0.029	153.0	0.11	4.0	4.0	4.4	4.0	120
75	0.050	126.8	0.20	4.1	3.9	4.8	4.1	120
75	0.053	229.8	0.23	4.3	4.0	4.4	4.2	90
80	0.021	29.1	0.11	4.9	4.4	4.3	4.4	5
80	0.028	3.0	0.12	4.1	3.9	4.0	3.9	0.5

Also the maximum observed lethalities (lethality is in unit of $\log_{10}$) and the max time of measurements are given.

Table 3 presents the average $\log_{10}$ reductions of spores for the two-grill types, preparations and target internal temperatures. There are two clear patterns: 1) For non-heat- shocked spores, for all comparisons, the average reductions were greater by about an average of 1 $\log_{10}$ for the gas grill compared to the corresponding average reductions associated with the electric grill; and 2) the reductions for stock A spores were larger, on average, than those for stock B spores. In addition, in all but one case, the $\log_{10}$ reductions for the non- heat -shocked spores were on average less than or equal to corresponding reductions for the heat-shocked spores. The one exception was for the gas grill, stock B spores. Comparing the impact of the different grills for heat-shocked spores, for most cases there were not statistically significant differences between reductions; however, for the high temperature treatment (93.3 °C), for the stock A spores, the ½ $\log_{10}$ difference between the 3.48 $\log_{10}$ reductions obtained for the gas grill versus 2.97 $\log_{10}$ reduction obtained for the electric grill suggests the possibility of an interaction effect of preparation, temperature and grill type on the inactivation kinetics. These results suggest that the type of grill used might have an effect on lethality, depending upon whether or not the spores were previously heat-shocked if the temperatures are not high, but not otherwise.

Thus, in summary, it appears that there could be a complex interaction taking place, where relative reductions depend upon the spore stock, whether spores are heat shocked or not, and the type of grill. We assume, but have no substantiating data, that temperatures were appreciably higher on the surface of patties where the meat was in direct contact with the heat. As such, we also assume that the observed reductions are primarily due to death of spores on the surface of the patties and that surviving and injured spores were mostly recovered from within the meat wherein the temperature was not as high for as long and wherein the fat content and other intrinsic factors may have afforded some protective effects to the spores. As mentioned above, the times to reach targeted internal temperatures for the gas grill were larger than the times for the electric grill. This longer time could thus result, on average, in a greater exposure to heat over a period time thus resulting in greater reductions of spores, particularly for the more heat resistant, non-heat-shocked, spores. Such assumptions are a likely starting point for further studies. Lastly, no viable spores of *B. anthracis* Sterne of either spore stocks were recovered by direct plating (≤1.3 $\log_{10}$ CFU/ml) the purge from ground beef patties that were inoculated with heat-shocked and non-heat shocked spores and cooked on either of the commercial grills.

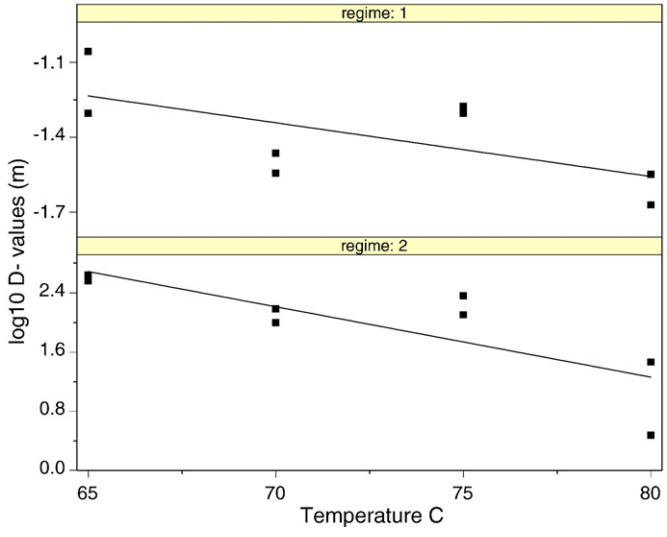

Fig. 2. Thermal death curves ($\log_{10}$(*D*-values) versus time) for the two time regimes identified from a bi-phasic survival curve model.

The literature provides sufficient documentation that the sporulation medium and the times and temperatures used for spore preparation and sub-lethal heat shock, and/or the heating menstruum can collectively affect spore resistance to heat (Beaman, Pankratz, & Gerhardt, 1988; Byrne et al., 2006; Foegeding & Busta, 1981; Ocio, Fernández, Rodrigo, & Martinez, 1996; Raso, Palop, Bayarte, Condón, & Sala, 1995). These data of our study indicate that the source of spore stock, the type of cooking, and whether or not spores were heat shocked may affect the subsequent heat stability of the spores during cooking. Further, the results presented herein suggest that spores of the Sterne strain of *B. anthracis* do not possess any unique characteristics compared to spores of other *Bacillus* species, such as *B. cereus* and *B. subtilis*, which would predispose them to greater or less survival under normal cooking parameters used for ground beef patties (Murray, 1931).

Heat resistance of bacterial spore-formers has been correlated with DNA content, dipicolinic acid (DPA) levels, calcium chelates with DNA, mineralization and dehydration, as well as with thermal adaptation of the spore core (Beaman & Gerhardt, 1986; Belliveau, Beaman, & Gerhardt, 1990; Bender & Marquis, 1985; Lindsay & Murrell, 1985; Palop et al., 1999). The composition and pH of the medium, nature of acidulants, the presence of inhibitors, and temperature and time of incubation can also significantly affect survival counts and, consequently, the values obtained for the parameters used to characterize heat resistance (Foegeding & Busta, 1981; Russell, 1982). Scott & Bernard (1982) suggested that there may be significant variations among different strains and among subcultures of the same strain relative to the reported thermal death time values. Our data validate that the source of the spore crop may appreciably affect the subsequent thermal stability of the spores in cooked ground beef.

The heat resistant nature of *B. anthracis* spores makes this pathogen a potential public health hazard in ground beef patties, particularly those that may be consumed either rare [internal temperature of ca. 60 °C (140 °F)] or medium-rare [internal temperature of ca. 63 °C (145.4 °F)] (Ralston, Brent, Starke, Riggins, & Jordan Lin, 2002). In the present study, cooking 95-g ground beef patties on commercial grills, resulted in reductions of ca. 0.8 to 3.5 $\log_{10}$ for spore stock A and B of *B. anthracis* Sterne after heating to 71.1 °C (160 °F), 82.2 °C (180 °F), or 93.3 °C (200 °F) on either the open-flame gas grill which required ca. 9.6 min to reach the target internal temperatures or on the clamshell electric grill which required ca.

Table 3
Reduction (log_{10} CFU/g) of heat shocked (HS) and non-heat shocked (NHS) spore stocks A and B of *Bacillus anthracis* Sterne in ground beef patties (95 g) that were cooked using two different commercial grills[1].

Spore preparation/ spore treatment	Gas open-flame grill temperature				Electric clamshell grill temperature			
	Before cooking	71.1 °C (160 °F)	82.2 °C (180 °F)	93.3 °C (200 °F)	Before cooking	71.1 °C (160 °F)	82.2 °C (180 °F)	93.3 °C (200 °F)
A								
HS	5.74 (±0.03)	2.86^{abcde,b} (±0.19)	2.91^{abcde} (±0.20)	3.48^{a} (±0.11)	5.63 (±0.12)	2.95^{abcd} (±0.08)	2.92^{abcde} (±0.12)	2.97^{abc} (±0.09)
NHS	5.79 (±0.03)	2.80^{abcdef} (±0.16)	2.91^{abcde} (±0.16)	3.16^{ab} (±0.56)	5.83 (±0.12)	2.08^{cdefgh} (±0.21)	1.99^{defghi} (±0.27)	1.96^{efghi} (±0.34)
B								
HS	5.23 (±0.64)	0.78^{k} (±0.16)	1.08^{ijk} (±0.30)	1.57^{ghijk} (±0.18)	5.89 (±0.10)	0.98^{jk} (±0.32)	1.32^{hijk} (±0.42)	1.89^{fghij} (±0.39)
NHS	6.02 (±0.06)	1.83^{ghij} (±0.14)	2.49^{bcdefg} (±0.32)	2.86^{abcdef} (±0.75)	6.11 (±0.10)	0.93^{jk} (±0.16)	1.20^{hijk} (±0.07)	1.80^{ghij} (±0.15)

[1]Mean of three trials (±standard deviation) ($N = 3$ trials; $n = 3$ samples per trial).
[2]Means with the same letters among the rows and columns are not significantly ($P > 0.05$) different.

4.0 min to reach the target internal temperatures. Further, we observed about a 0.8 to 2.9 log_{10} reduction of spores in beef patties at target temperatures that included the USDA/FSIS recommended temperature of 71.1 °C (U.S.D.A., 1998). In comparison, our data using a water bath system and heating at 65 to 80 °C predict nearly 4 log_{10} reductions in spore levels for short times, ~½ min, depending possibly on the temperature. The disparity between these findings leads us to conclude that the water bath system probably does not provide a reasonable simulation of the environment that would exist for spores in beef when cooked on commercial grills. Thus models developed from such controlled experiments would not provide a reasonable predictor of results likely to be obtained when cooking on commercial grills. Further experiments for actual cooking scenarios are needed to develop better models for prediction that take into account the heat flow dynamics involved with cooking. The heat dynamics involved with cooking need to be understood better before results obtained in a model system, such as 3 g portions of irradiated ground beef heated in a circulating water bath, can be used to help develop models for lethality when cooking on commercial grills. However, in the present study, we did validate that cooking ground beef patties on a commercial grill at a temperature considered to be "well-done" and a temperature recommended by the USDA/FSIS, that being a target instantaneous internal temperature of 71.1 °C (160 °F), is sufficient to appreciably lessen the potential risk of illness associated with this spore-forming bacterium in the event of its purposeful addition to raw ground beef.

Acknowledgments

We extend our appreciation to the following individuals at the USDA/ARS/ERRC in Wyndmoor, PA, who in large measure contributed to the successful completion of this study by sharing their time, talents, resources, and opinions: Brad Shoyer, Rosemary Martinjuk, John Cherry, Angie Martinez, Ellen Sanders, John Phillips, and the "ERRC Bio-Security Special Projects Team". For these same reasons, we are extremely grateful to our colleagues from the USDA/FSIS, namely Lynda Kelley and Robert Phillips (Athens, GA) and Tim Mohr (Omaha, NE). This research was supported, in part, by the National Biodefense Analysis and Countermeasures Center, the USDA/FSIS, and the USDA/ARS.

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